DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection on 5 March 2010. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed 5 March 2010 has been entered wherein no claim was amended or cancelled, but arguments traversing the rejections of record were presented. Claims 1 and 15-18 remain in the application and are examined herein.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 USC § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1 and 15-18 remain rejected for reasons of record under 35 USC § 103(a) as being unpatentable over Estell et al., **US 7,332,320**, and Roggen et al., **US 2005/0181446**,, in view of Bryan et al., **US 4,980,288**, and Hastrup et al., **US 5,741,694**, all of record.

Applicant's arguments at page 3 the Response filed 5 March 2010 have been fully considered but are not persuasive. Applicant's suggests that there is insufficient motivation in the combination of prior art cited in the rejection of record to make a subtilisin GG36, which will also be a subtilisin 309 variant, that comprises either set of amino acid substitutions recited in claim 1, i.e., a V26T+N218S substitution pair or a V16S+N218S substitution pair, and that also has the properties recited in claim 1, i.e., "protease activity under detergent wash conditions" and "improved thermostability as compared to the wild-type GG36". Applicant particularly urges that "nothing in Estell et al. [ ] would lead one of skill in the art to produce the presently claimed variants that have protease activity in detergents and are thermostable." Applicant does not dispute that it has long been well known in the art of subtilisin modification that the N218S substitution alone confers enhanced thermal stability, thus improved wash performance, as a component detergent compositions wherein it is formulated upon any subtilisin in which it is introduced by comparison with, e.g., the thermostability of precursor subtilisins lacking this substitution, at temperatures exceeding the temperature indicated in Table 4 of Applicant's

specification. Indeed, both Bryan et al. **'288** and Hastrup et al. **'694** were cited in the rejection of record as evidence of the recognition of the value of an N218S substitution. While Bryan et al. **'288** were not the first to teach incorporating the N218S substitution in a subtilisin they were the first to measure the degree of thermostability this substitution provides in a subtilisin having protease activity under detergent wash conditions. In particular, Bryan et al. **'288** teach that this substitution is advantageously made in subtilisins generally at col. 6, lines 9-23, and that it "enhances the thermal stability of subtilisin" at col. 8, lines 10-20. Bryan et al. **'288** also teach that their singly-substituted GX7150 subtilisin variant – which is the amino acid sequence of SEQ ID NO:3 herein modified only by the N218S substitution – has "almost four times [the proteolytically active half-life] of the wild type" after incubation at 65°C when measured over a period of time exceeding 13 hours and further teach that this "single amino acid change . . . dramatically increases the kinetic thermal stability of subtilisin." See col. 12, lines 36-68, col. 13, lines 38-56, and Figure 4. Bryan et al. **'288** additionally teach that the N218S substitution provides thermally stabilizing advantages in a variety of environments, wherein the catalytic activity of the variant is prolonged relative to the catalytic activity of the wild type subtilisin in detergents and in actual wash conditions comprising "15 minutes at 75 RPM agitation at 55°C". See col. 13, line 69, through col. 15, line 19, particularly the results of Table I at col. 15, and Figures 5-7. Bryan et al. moreover teach that the N218S substitution is the primary contributor to the thermostability of several multiply substituted subtilisin variants. See col. 15, line 49, through col. 16, line 19, including the results of Table II, and Figure 8. Because Bryan et al. did not extend their teachings to introduction of the N218S substitution in the subtilisin having the amino acid sequence of SEQ ID NO:6 herein, termed a GG36 subtilisin by Applicant but also known in the art as subtilisin 309 and marketed under the trade name Savinase™, the teaching of Hastrup et al. **'694** of introducing the N218S substitution in the amino acid sequence of subtilisin 309 is now cited. Numbering the position as 218 by correspondence with the amino acid sequence of subtilisin BPN', which is the amino acid sequence of SEQ ID NO:3 herein, Hastrup et al. **'694** designate the singly-substituted variant as "f) Asn-218 Ser" in their list of mutations at col. 21, and teach that their N218S variant has significantly increased washing ability by comparison with the wild type subtilisin 309 in actual wash conditions comprising the detergent composition and the assay conditions taught at col. 15, line 64, through col. 16, line 44, for a duration of 10 minutes at 100 rpm, providing the results in Table VI of Example 6.2.6 at col. 25, lines 15-40. Hastrup et al. **'694** further teach that a subtilisin 309 variant having only the N218S substitution exhibits significantly enhanced thermostability with the same detergent composition and same duration of wash at 100 rpm when "tested against the wild type enzyme"

at 40°C and a two-fold enhanced thermostability by comparison with the wild type enzyme at 60°C in Table VII of Example 6.2.7 at col. 25, lines 41-63.

Clearly, ample motivation existed long before the invention was made to make an N218S substitution in a subtilisin G36/subtilisin 309 amino acid sequence and Applicant presents no evidence that combining an N218S substitution with either of a V26T or a V26S substitution in any subtilisin might somehow detract from the advantages conferred by the N218S substitution, advantages that meet the functional limitations of claim 1. Indeed, Estell et al. were cited in the rejection of record for teaching preparation of subtilisin variants with reduced immunogenicity, wherein a valine present at a position 26 in a generic subtilisin, where the position is numbered according to the amino acid sequence of subtilisin BPN' which is SEQ ID NO:2 herein, is replaced by serine or threonine, e.g.,"V26S, V26T", and wherein an asparagine present at position corresponding to position 218 in the amino acid sequence of subtilisin BPN' is replaced by serine, i.e., "N218S", where the allergenicity-reducing amino acid substitutions are taught to be combined, in their Example 6, with "mutations known in the art to effect altered thermal stability . . . and/or altered pH (e.g., alkaline) stability of proteins" including the N218S amino acid substitution and that "[a]fter the variants are produced, they can be screened for the desired property (e.g., altered or low or reduced immunogenic response, increased thermal or alkaline stability, etc.)". See, e.g., col. 9, lines 42-50, the paragraph spanning cols. 9 and 10, vol. 10 lines 16-59, the paragraph spanning cols. 11 and 12, and, particularly, the first full paragraph of col. 12, col. 15, lines 30-36, col. 26, lines 3-15 and 40-50, and the paragraph spanning cols. 26 and 27. Note also, that Estell et al. consider that epitope regions in other subtilisins may "correspond" to those in subtilisin BPN', including those "corresponding to residues 25-39". See col. 16, at lines 3-5. Thus, the issue presented is whether or not one of ordinary skill in the art at the time the invention was made would have recognized that the teaching of Estell et al. of combining allergenicity-altering mutations as well as ancillary, stability modulating, substitutions, particularly N218S, in generic subtilisins could usefully be extended to a subtilisin G36/subtilisin 309 amino acid sequence where Estell et al. teach that the N218S substitution is among a select set of eight "Lower Allergenicity Stabilizing Mutations". See their Example 6 at cols. 51 and 52.

Thus Roggen et al. were cited for teaching the preparation of variants of the wild-type "savinase" subtilisin having the amino acid sequence of their SEQ ID NO:24, a sequence that is entirely identical to the amino acid sequence of SEQ ID NO:6 herein, wherein a serine or threonine is substituted for the valine at the subtilisin BPN'-correspondent 26 to provide a

variant subtilisin having modified immunogenicity. See, e.g., the "savinase" epitope regions "sav4.0", "sav14.0", "sav.16.0", "sav17.0", and "sav18.2" in Table 2 at pages 54-55, as well as claims 76 and 80-85, particularly claim 80.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to prepare a subtilisin variant of claim 1 herein having "protease activity under detergent wash conditions" as well as having "improved stability as compared to [the] wild-type GG36" by selecting the "savinase" amino acid sequence of SEQ ID NO:24 of Roggen et al. identical to the GG36 subtilisin amino acid sequence of SEQ ID NO:6 herein to prepare a variant subtilisin exhibiting an altered immunogenicity by introducing either of the V26S or V26T substitutions that are taught by both Estell et al. and Roggen et al. and to further introduce an N218S substitution, i.e., at the position corresponding to position 218 of SEQ ID NO:3 herein, in order to provide a dually-substituted variant according to claim 1 herein having both protease activity under wash conditions and improved thermostability by comparison with the wild-type subtilisin 309/GG36, meeting the limitations of claim 1 herein. This is because

(i) Estell et al. teach that the region present in subtilisins generally comprising the subtilisin BPN'-correspondent position 26 contributes to the generation of a T-cell response in animals and that replacing the valine in this region with serine or threonine will alter the immunogenicity of the variant by comparison with that of the native subtilisin,

(ii) Roggen et al. agree with Estell et al. in particularly identifying the valine at the position 26 in SEQ ID NO:6 herein as contributing to an epitope and teaching, as in their claim 80, that it may be advantageously replaced with a serine or threonine,

(iii) Estell et al. further teach that the N218S substitution is among a preferred set of stabilizing substitutions that can advantageously be combined with an amino acid substitution that alters the immunogenicity of a variant subtilisin relative to the precursor,

(iv) such an artisan would readily recognize that the asparagine at position 212 in the amino acid sequence of SEQ ID NO:6, which is also the amino acid sequence of subtilisin 309 as taught in the prior art, corresponds to the asparagine at position 218 in the amino acid sequence of subtilisin BPN' according to Estell et al., and

(v) such an artisan would be well aware that Bryan et al. had already replaced the asparagine with a serine in subtilisin BPN', and Hastrup et al. had already replaced the asparagine with a serine in subtilisin 309/GG36 subtilisin, to provide enhanced thermostability in both subtilisins.

It would further have been obvious to one of ordinary skill in the art at the time the invention was made to prepare the DNA molecule, expression vector, host cell, and cleaning composition of claims 15-18 herein because Estell et al. teach (1) that it is advantageous to make such variant subtilisins by preparing DNA molecules encoding such variants, expression vectors comprising such variant-encoding DNA molecules, and host cells comprising such expression vectors in order to practice a method of making such variants utilizing such host cells and (2) that subtilisin variants with altered immunogenicity are advantageously incorporated in cleaning compositions. "The question is not whether the combination was obvious to the patentee but whether the combination was obvious to a person with ordinary skill in the art. Under the correct analysis, any need or problem known in the field of endeavor at the time of invention and addressed by the [prior art] can provide a reason for combining the elements in the manner claimed." *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1742, 82 USPQ2d 1385, 1398 (U.S. 2007). Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success in practicing the claimed invention, thus the rejection of record is maintained.

This application currently names joint inventors. In considering patentability of the claims under 35 USC § 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 USC § 103(c) and potential 35 USC §§ 102(e), (f) or (g) prior art under 35 USC § 103(a).

Conclusion

All claims are drawn to the same invention claimed in the application prior to the entry of the submission under 37 CFR 1.114 and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the application prior to entry under 37 CFR 1.114. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action after the filing of a request for continued examination and the submission under 37 CFR 1.114. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 571.272.0933 and whose FAX number is 571.273.0933. The examiner can normally be reached Monday through Friday between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisory Primary Examiner, Manjunath Rao, can be reached at 571.272.0939. The official FAX number for all communications for the organization where this application or proceeding is assigned is 571.273.8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571.272.1600.

/William W. Moore/
Examiner, Art Unit 1656

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